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THE EFFECT OF CO_2 LASER ON FIBROBLAST CULTURES

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Surgeons, particularly in the field of otolaryngology/head and neck surgery, have been developing and refining applications for the carbon dioxide (CO_2) laser for the past 10 to 15 years. During this time, many studies of CO_2 laser wound healing have appeared, most comparing healing of laser and standard scalpel incisions. These reports consistently described delays in CO_2 laser wound healing when compared to healing of scalpel incisions. Several studies involving skin wound tensile strength comparisons between scalpel and laser incisions in rats (6) and pigs (2,5) have shown that healing laser wounds are significantly weaker for several weeks post-incision. In a histologic comparison of laser and scalpel incisions in pigs, Norris and Mullarky (10) noted delayed collagen formation in laser incisions as compared to scalpel incisions. Using laser- and scalpel-cut explants taken from pig skin, Moreno et al (9) found that while epithelialization proceeded at the same rate from both types of explant, the onset of epithelialization from laser-cut explants was delayed. Despite this accumulation of information on CO_2 laser wound healing, a good understanding of this topic as well as the effects of CO_2 laser on cell growth and metabolism has not yet been achieved. Our study investigated the CO_2 laser wound healing process by examining laser effects on fibroblasts, perhaps the most important cells involved in the wound healing process. Specifically, we studied the effect of noncytotoxic, defocused CO_2 laser energy, similar to that experienced by cells adjacent to a surgical laser wound, on proliferation of human dermal fibroblasts in culture.

Materials and Methods

The basic method used in this experiment is based on that used by Castro et al (4) in a previous Nd/YAG laser experiment.

Preparation of cell cultures

Frozen samples of human dermal fibroblasts were thawed and plated in culture flasks containing a growth medium composed of Dulbecco's modified Eagle's medium (DMEM) with 20% fetal bovine serum, 100 U/ml penicillin, 100 ug/ml streptomycin, and .025 ug/ml amphotericin. Cells were then removed by trypsinization and suspended for inoculation into 96-well tissue culture plates (Gibco, diameter of single well = .64 cm). Prior to inoculation, the suspension was counted by hemacytometer and the volume then adjusted to allow inoculation of 5×10^3 cells in .2 ml medium per well. Pilot experiments demonstrated that cells reached confluence at approximately 1×10^5 fibroblasts per well as determined by Coulter counter, following trypsinization. Since fibroblasts typically divide every 12 to 24 hrs. until reaching confluence and subsequent growth inhibition, inoculation of cells at less than 10% of confluence assured that cells would be in the logarithmic growth phase throughout the experiment. Cells were allowed 24 hrs. to attach prior to laser treatment. Immediately prior to irradiation, the medium was removed to minimize energy absorption by the growth medium, thereby limiting possible thermal effects due to laser heating of the growth medium. After laser treatment, culture wells received .2 ml of fresh medium and were then returned to incubation at 37° C. Culture medium was changed every 2 days.

Laser properties

The laser used in this experiment was a Sharplan 1040 surgical CO_2 laser with milliwatt capabilities (100 mW and up). Culture irradiation was performed using continuous wave mode. We adjusted beam diameter using the continuously variable defocus dial (CVD) on the Sharplan 719 micromanipulator attachment. Using a 400 mm focal length lens, adjustment of the CVD allowed us to produce a .616 cm diameter spot. This spot diameter allowed coverage of 92.6% of the culture surface, thereby producing extensive coverage of

the culture yet allowing room to avoid interference of the beam by the sides of the well.

Selection of Beam Intensities

Information on laser-tissue interactions published by Sliney (12) suggested that the maximal noncytotoxic CO_2 beam intensity was in the range of 10 W/cm^2 and less (precise CO_2 laser dose-response curves using fibroblast cultures are presently being established in our laboratory). We therefore chose to irradiate cultures using power levels from 0.1 to 3.0 W., holding spot diameter constant at .616 cm (Table 1). Exposure duration was held constant at .5 seconds.

Determination of cell viability and proliferation

At 1, 12, 24, 48, and 72 hrs. post-irradiation, cells from 4 wells per each power setting along with 4 control wells were examined for viability and proliferation using the trypan blue exclusion test and hemacytometer counting. Preparation of each well for the trypan blue exclusion test involved removal of growth medium, followed by a single wash using .2 ml of phosphate buffered saline (PBS) warmed to 37° C. Cells were then trypsinized for 3 minutes using .1 ml of .25% trypsin in Hank's salts with EDTA. A .1 ml aliquot of growth medium warmed to 37°C was then added to the well, raising the volume of cell suspension to .2 ml. In a separate vial, a .1 ml sample of cell suspension was added to .2 ml of growth medium, followed by the addition of .025 ml trypan blue, to produce the final sample used for viability examination and hemacytometer counting. For all wells, the number of viable cells at a particular time point was calculated from the average of 4 wells.

Statistical Method:

Data were analyzed statistically using one way analysis of variance.

Results

The actual data are shown in Table 2. Each cell count shown represents a mean of 4 sample wells per energy and time point. Note first that all cells appear to remain in the lag phase of growth until some time after 48 hrs. post-inoculation. The normal lag phase for most cells lasts approximately 24 hrs. post-inoculation. Therefore, a separate experiment was performed to study the effect. Cultures from the same stock and cell line as those used in this experiment were grown under identical conditions (with the exception of being radiated), and their normal growth behavior observed. Indeed this particular line of fibroblasts consistently showed a 48 hr. lag phase post-inoculation, demonstrating this to be a normal variation for this individual cell line.

Figure 1 summarizes the data. Compared to sham-treated controls, cells treated with 3.0 W (intensity = 10.1 W/cm^2) demonstrated a significant (P 0.01) decrease in rate of cell growth when compared to the control, non-irradiated group 0.1 W (intensity = .336 W/cm^2) had significantly (P 0.02) accelerated growth rate. Cells treated with 0.6, 1.2, 1.8 and 2.4 W had growth rates statistically similar to control cells.

Discussion

The purpose of this experiment was to study fibroblast response to noncytotoxic CO_2 laser energies. In particular, we were interested in responses to energy levels experienced by viable fibroblasts adjacent to laser wounds. These would presumably be the fibroblasts involved in wound repair.

Our results demonstrate that CO_2 laser energy delivered at intensities in the range of 10 W/cm^2 has an inhibitory effect on fibroblast proliferation.

This suggests that the well-characterized delay seen in CO_2 laser wound healing is due, at least in part, to inhibited fibroblast proliferation. Several explanations for this effect are possible. A thermal effect caused by heating of intracellular water may have caused a generalized disruption in cell metabolism. The laser could also be exerting specific inhibitory effects on individual processes associated with cell division, one example being DNA synthesis. Further studies examining the effect of these CO_2 beam intensities on fibroblast DNA synthesis and collagen production are now underway and will hopefully clarify the nature of the effect demonstrated in this experiment.

This ability to inhibit fibroblast proliferation within the range of maximum noncytotoxic laser intensities suggests that the CO_2 laser would be quite useful in situations where minimal postoperative scarring is desired. One such instance in which a CO_2 laser is already often used as the method of choice is in treatment of vocal chord lesions. Postoperative vocal chord fibrosis with residual impairment in chord function is a complication that could potentially be eliminated by optimal use of the CO_2 laser. Pending the development of flexible fiberoptic endoscopes capable of transmitting the CO_2 wavelength, CO_2 laser vaporization could become an ideal method for treatment of esophageal strictures with a minimal rate of stricture recurrence.

In contrast to the growth inhibiting effect discussed above, CO_2 laser irradiation at an intensity of .336 W/cm^2 produced an increased rate of fibroblast proliferation compared to sham-treated controls. Potential explana-tions could include a proliferation-enhancing thermal effect or perhaps specific effects on processes involved in cell division.

Possible enhancement of would healing and cell metabolism using low dose laser radiation has been studied by a number of investigators using lasers other than CO_2 (1,3,7,8,11). The results of our experiment suggest

that low dose CO_2 laser irradiation could potentially be used to accelerate the wound healing process. In this manner, the CO_2 laser could be used to enhance wound healing in patients with impaired wound healing ability, including elderly, nutritionally deficient, diabetic, and steroid-dependent patients.

While the results of this experiment are interesting, further studies regarding the nature of CO_2 laser effects on fibroblasts are needed before this information can be considered for practical use. In currently ongoing experiments, we plan to measure the effect of CO_2 laser energies on fibroblast collagen synthesis in tissue culture. Indeed future developments within the broader field of laser-cellular interactions will certainly present many new, therapeutically beneficial options involving the use of lasers.

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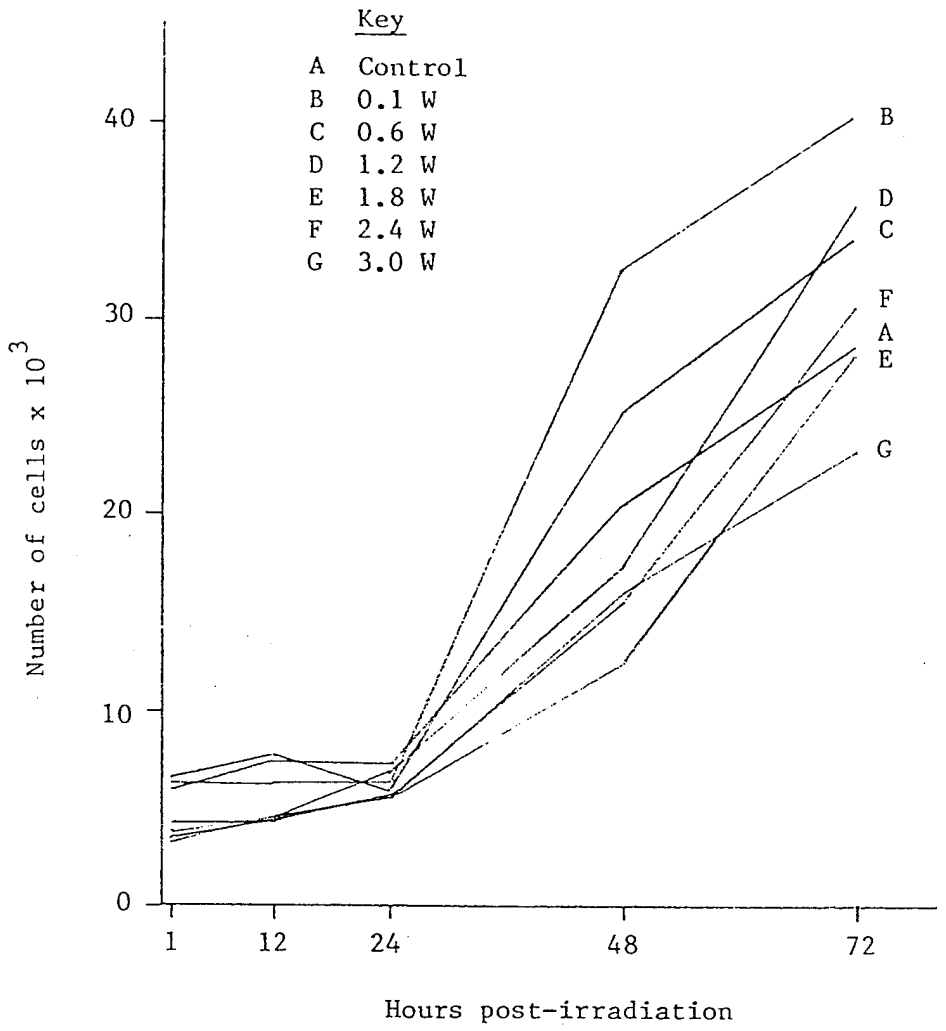

Figure 1. Effect of CO_2 laser irradiation on human dermal fibroblast proliferation

TABLE 1. Intensity and Energy Density Delivered to Fibroblasts at Six Different Power Settings

Power Setting (W)	Intensity (W/cm^2)	Incident Energy Density ($Joules/cm^2$)
.1	.336	.168
.6	2.01	1.01
1.2	4.03	2.01
1.8	6.04	3.02
2.4	8.05	4.03
3.0	10.1	5.03

* spot diameter .616 cm

* exposure duration .5 sec

TABLE 2: Effect of CO_2 Laser Irradiation on Human Dermal Fibroblast Proliferation (cells/well)

Power Setting (W)	Intensity (Wcm^2)	Hours Post-irradiation 1	12	24	48	72
0	CONTROL	5938 ± 51	7500 ± 62	7188 ± 60	20469 ± 190	28594 ± 130
0.1	.336	6250 ± 58	6250 ± 59	6563 ± 60	32500 ± 141	40313 ± 146
0.6	2.01	6563 ± 45	7813 ± 48	5938 ± 50	25313 ± 137	34063 ± 150
1.2	4.03	3750 ± 37	4688 ± 35	6875 ± 52	17188 ± 125	35938 ± 138
1.8	6.04	3125 ± 29	4688 ± 30	5625 ± 45	12188 ± 128	28438 ± 133
2.4	8.05	3438 ± 38	4375 ± 35	5313 ± 51	15313 ± 133	30625 ± 139
3.0	10.1	4063 ± 38	4063 ± 31	5625 ± 55	15938 ± 131	23125 ± 132

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